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TITLE: Colon cancer KH-1 and N3 antigens

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Brief Description of Drawings Paragraph (2):
[0025] FIG. 1 show the structure of the cell surface antigen KH-1 ceramide and its bioconjugateable O-allyl ether form.

Detail Description Paragraph (113):
[0149] The present invention also provides a method of preparing a protected nonasaccharide ceramide having the structure: 52

Detail Description Paragraph (119):
[0155] (c) reductively acylating the azide intermediate with palmitic anhydride under suitable conditions to form a protected nonasaccharide ceramide;

Detail Description Paragraph (120):
[0156] (d) reducing the protected nonasaccharide ceramide formed in step (c) under suitable conditions to form a deprotected nonasaccharide ceramide;

Detail Description Paragraph (121):
[0157] (e) acylating the deprotected nonasaccharide ceramide under suitable conditions to form an acylated nonasaccharide ceramide; and

Detail Description Paragraph (122):
[0158] (f) saponifying the acylated nonasaccharide ceramide under suitable conditions to form the nonasaccharide ceramide. The invention encompasses the method wherein the oxygen transfer agent is DMDO. The invention also encompasses the method wherein the conditions of the coupling step comprise ZnCl.sub.2. The method further encompasses use of an azide intermediate which is reductively acylated in step (c) in the presence of Lindlar's catalyst. The invention further encompasses the method wherein conditions of the saponifying step comprise MeONa in methanol.

Detail Description Paragraph (225):
[0237] Antisera at 1:1500 dilution or mAb BR-96 at 0.1 .mu.g/ml were mixed with various concentrations of structurally related and unrelated carbohydrate antigens. The mixture was incubated at room temperature for 30 min, and transferred to an ELISA plate coated with KH-1-ceramide. ELISAs were performed as described above. Percentage inhibition was calculated as the difference in absorbance between the uninhibited and inhibited serum.

Detail Description Paragraph (227):
[0239] Groups of mice (CB6F1 female; 6 weeks of age) obtained from Jackson Laboratory, Bar Harbor, Me., were immunized subcutaneously with KH-1-KLH or KH-1-M.sub.2C.sub.2H-KLH containing equivalent to 3 .mu.g KH-1 only (the quantity of KLH varied depending on the epitope density) mixed with 10 .mu.g of immunological adjuvant QS-21, a saponin derivative from the bark of the Quillaja saponaria Molina tree (Aquila,. Worcester, Mass.) at 0, 1 and 2 weeks and bled 10 days after the third immunization. The presence of antibody was assayed by an enzyme linked immunosorbent assay (ELISA) as described in Kensil C. R. et al., J. Immunol., 1993, 146, 431-437, using KH-1 ceramide as target antigen. The cell surface reactivity of anti-KH-1 antibodies was tested on KH-1 positive MCF-7 cells by flow cytometry assays. The mice

vaccinated with KH-1-M.sub.2C.sub.2H are made the high titer antibody against the synthetic KH-1 and the antibodies were reacted strongly on the cell's surface that expressed KH-1 antigens.

Detail Description Paragraph (229):
[0241] 0.5 .mu.g KH-1 ceramide and other Ley antigen and unrelated antigens were spotted on nitrocellulose strips. Dot blot Immune staining was performed monoclonal antibody BR 96 after blocked with 6% bovine serum albumin in PBS for 1 h and incubated with antibody BR 96 (diluted 1:500 in PBS) overnight at room temperature. The strips were washed with PBS containing 0.05% Tween 20 and incubated with anti-mouse IgG antibody conjugated with horseradish peroxidase at 1:200 dilution for 3 h at room temperature. Then the strips were washed with PBS-0.05% Tween 20 and developed with 4-chloro-1-naphtol-H.sub.2O.sub.2. The results are summarized in Table 1. The synthetic KH-1 reacted very strongly when compared with other Le.sup.y related antigens unrelated antigens were failed to react with BR 96 antibody.

Detail Description Paragraph (237):
[0249] Thus, it was possible to introduce the three .alpha.-L-fucose residues in one step via donor 14 (Danishefsky, S. J.; Gervay, J.; Peterson, J. M.; McDonald, F. E.; Koseki, K.; Oriyama, T.; Griffith, D. A.; Wong, C. -H.; Dumas, D. P., J. Am. Chem. Soc., 1992, 114, 8331), thereby affording a 60% yield of the nonasaccharide. From 15, the sorts of protocols required to reach 1 and 2 were qualitatively well precedented. In the case of 2, the chemistry followed very closely from the methodology developed for the globo-H breast tumor, conjugatable allyl glycoside. M. T. Bildoeau, T. K. Park, S. Hu, J. T. Randolph, S. J. Danishefsky, P. O. Livingston, and S. Zhang, J. Am. Chem. Soc., 1995, 117, 7840; T. K. Park, I. J. Park, I. J. Kim, S. Hu, M. T. Bilodeau, J. T. Randolph, O. Kwon and S. J. Danishefsky, J. Am. Chem. Soc., 1996, 118, 11488. To reach the naturally occurring glycolipid antigen 1, a small but useful variant was introduced wherein the pre-ceramide acceptor 17 was coupled to an anomeric thioethyl donor derived from the glycal epoxide. For a review, see: Fugedi, P.; Garegg, P. J.; Lnn, H.; Norberg, T.; Gycocnjugate J., 1987, 4, 97; Lnn, H., Carbohydr. Res., 1985, 139, (105) 115; Lnn, H., Carbohydr. Chem., 1987, 6, 301.

Detail Description Table CWU (3):
3TABLE 3 Binding of Monoclonal Antibody Br 96 with KH-1 and other Carbohydrates by Dot-blot. Monoclonal Antibody BR 96 (Le.sup.y F12 Carbohydrate related) (FucosylGM1) KH-1 ceramide very strong negative (+++) Le.sup.y-ceramide strong (++) negative Le.sup.y-KLH strong (++) negative Globo H ceramide negative negative TF-ceramide negative negative SSEA-ceramide negative negative Le.sup.y/Le.sup.b (Ovarian cyst Mucins-Tighe)* strong (++) negative Le.sup.a/Le.sup.x (Ovarian cyst mucins-N1)* weak (+) negative Non fucosylated precursor of Lewis* negative negative Le.sup.a-PAA negative negative Le.sup.x-PAA weak (+) negative FucGMI negative very strong (+++) GD3 negative negative *extracted from patient tissue

CLAIMS:

70. A method of preparing a protected nonasaccharide ceramide having the structure: 117which comprises: (a) epoxidizing a protected nonasaccharide having the structure: 118with an oxygen transfer agent under suitable conditions to form a protected nonasaccharide epoxide; (b) coupling the protected nonasaccharide epoxide formed in step (a) with an azide having the structure: 119under suitable conditions to form a nonasaccharide azide intermediate; (c) reductively acylating the azide intermediate with palmitic anhydride under suitable conditions to form a protected nonasaccharide ceramide; (d) reducing the protected nonasaccharide ceramide formed in step (c) under suitable conditions to form a deprotected nonasaccharide ceramide; (e) acylating the deprotected nonasaccharide ceramide under suitable conditions to form an acylated nonasaccharide ceramide; and (f) saponifying the acylated nonasaccharide ceramide under suitable conditions to form the nonasaccharide ceramide.